

## Novel bicyclic peptide plasma kallikrein inhibitors for the treatment of diabetic macular edema

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### Purpose

Plasma kallikrein (PKal) is a circulating protease which activity releases mediators of vasodilatation and vessel permeability. Vascular leakage plays a key role in diabetic macular edema (DME), a frequent complication in the progression of diabetic retinopathy and a major cause of vision loss. In an effort to develop new therapeutic modalities in the treatment of DME, we have identified bicyclic peptides which act as potent and selective inhibitors of PKal. The potential of these novel agents for the treatment of diabetes-related eye diseases was evaluated through in depth in vitro characterization as well as testing in in vivo preclinical models.

### Methods

Bicyclic peptides were obtained from linear peptides containing three cysteine residues followed by cyclization with a thiol-reactive molecular scaffold. Bicyclic peptides with PKal blocking activity were identified and enriched using the Bicycle Therapeutics proprietary phage display platform. Hits were further optimized for stability in biological matrices. PKal inhibition was measured in standard enzyme inhibition assays. Target specificity was evaluated by testing the bicyclic peptides against a panel of serine proteases. Inhibitory activity in vitreous and plasma was assessed by measuring the ability of the peptides to prevent release of the bradykinin in the presence of high molecular weight kininogen, a natural substrate of PKal. Stability in plasma and vitreous was measured by monitoring the chemical integrity of the peptides by LC-MS/MS. Pharmacokinetic properties in the rabbit eye were obtained by monitoring the peptide concentration in the vitreous by HPLC following intravitreal administration. Finally, bicyclic lead peptides were tested in a carrageenan-induced rat paw edema model and in a mouse model of diabetes-induced retinal permeability. Retinal permeability was assessed by the measure of the retinal fluorescence intensity following retrobulbar injection of fluorescein isothiocyanate-conjugated bovine serum albumin.

### Results

Screening large peptides-on-phage libraries, we identified highly potent and specific bicyclic peptide inhibitors of human PKal. Cross-reactivity against PKal from non-human species was more difficult to achieve, but nevertheless high enough for some peptides to be suitable for testing in e.g. rodent models. Stability of the bicyclic peptides in proteolytically-active biological matrices such as plasma was improved by rational design via the introduction of non-natural amino acids and non-peptidic bonds. Anti-PKal bicyclic peptides were shown to block the release of the bioactive peptide bradykinin in the presence of high molecular weight kininogen in both vitreous and plasma. Following intravitreal administration in rabbits, bicyclic peptides showed a long residence time in the eye, with a half-life of ~ 20 to 40 h. Two distinct bicyclic peptides were then tested in a rat model of carrageenan-induced paw edema. Intraperitoneal administration of either peptide led to a significant and dose-dependent inhibition of the carrageenan-induced paw edema. Finally, one selected bicyclic peptide was tested in a streptozotocin-induced diabetic model of retinal permeability. Retinal vascular permeability increased 3-fold in diabetic mice compared to non-diabetic control mice, and was significantly reduced following intravitreal administration of the selected bicyclic peptide.

### Conclusions

While significant progress in therapeutic management of diabetic retinopathy has been achieved, it remains the major cause of blindness in industrialized countries. Intravitreal administration of anti-VEGF

agents has been accepted as a primary therapy, and is usually effective in reducing macular edema and improving visual acuity. However, a significant proportion of the patients responds poorly or is refractory to the therapy. Inhibition of the pathways leading to edema downstream of VEGF or independent of VEGF therefore represents an attractive therapeutic opportunity. Constrained peptides allow the conformational locking of the bioactive conformation, resulting in higher affinity and specificity compared to the corresponding linear peptides. Combining the power of phage display with rationally-designed synthetic modifications has allowed the identification of bicyclic peptide PKA1 inhibitors that combine potency, specificity, in vivo stability and suitable pharmacokinetic properties. Efficacy of bicyclic peptides in a rat paw edema model and in a mouse model of diabetes-induced retinal permeability, demonstrated that the activity of these molecules is maintained in vivo, and further confirmed the postulated role of PKA1 in increased retinal permeability associated with diabetes mellitus. Our data thus demonstrate the potential of PKA1 inhibitory bicyclic peptides for the treatment of diabetes-related eye diseases.